

Claims

1. A method of identifying DNA responsible for conferring a particular phenotype in a cell which method comprises

5 a) constructing a cDNA or genomic library of the DNA of said cell in a suitable vector in an orientation relative to a promoter(s) capable of initiating transcription of said cDNA or DNA to double stranded (ds) RNA upon binding of an appropriate transcription factor to said promoter(s),

10 b) introducing said library into one or more of said cells comprising said transcription factor, and

15 c) identifying and isolating a particular phenotype of said cell comprising said library and identifying the DNA or cDNA fragment from said library responsible for conferring said phenotype.

2. A method according to claim 1 wherein said library is organised into hierarchical pools prior to step b).

3. A method of assigning function to a known DNA sequence which method comprises

25 a) identifying a homologue(s) of said DNA sequence in a cell,

20 b) isolating the relevant DNA homologue(s) or a fragment thereof from said cell,

25 c) cloning said homologue or fragment thereof into an appropriate vector in an orientation relative to a suitable promoter(s) capable of initiating transcription of dsRNA from said DNA homologue or fragment upon binding of an appropriate transcription factor to said promoter(s),

30 d) introducing said vector into said cell from

Sub
D2
CONT.
Sub A

step a) comprising said transcription factor, and
e) identifying the phenotype of said cell
compared to wild type.

5 4. A method according to any of claims 1 to 3
wherein said DNA library, homologue or fragment is
cloned in a sense and an antisense direction relative
to said promoter.

10 5. A method according to any of claims 1 to 3
wherein said DNA library, homologue or fragment is
cloned between two promoters capable of producing
dsRNA from said DNA library, homologue or fragment
upon binding of said transcription factor to said
15 promoters.

6. A method according to any of claims 1 to 5
wherein said cell is adapted to express said
transcription factor.

20 7. A method according to any of claims 1 to 6
wherein said DNA library, homologue or fragment is
constructed in a suitable vector which comprises a
sequence of nucleotides encoding said transcription
25 factor operably linked to a suitable promoter.

8. A method according to any of claims 1 to 6
wherein said transcription factor is encoded by a
further vector independent of the vector including
30 said DNA library, DNA homologue or fragment and which
sequence encoding said transcription factor is
operably linked to a suitable promoter.

35 9. A method according to claim 7 or 8
wherein said transcription factor comprises any of T7,
a

*Sub
P.A.
contd*

T3 or SP6 polymerase.

10. A method according to claim 7 or 8 wherein said suitable promoter comprises any of let 858, SERCA, UL6, myo-2 or myo-3.

11. A method according to any of claims 7 to 10, wherein said suitable vector or said further vector comprises a selectable marker.

10

12. A method according to claim 11 wherein said selectable marker comprises a nucleotide sequence capable of inhibiting or preventing expression of a gene in said cell and which gene is responsible for conferring a known phenotype.

15

20

25

13. A method according to claim 12 wherein said nucleotide sequence comprises a sequence which is a part of or identical to said gene conferring said phenotype, and which nucleotide sequence is itself oriented relative to a suitable promoter(s) capable of initiating transcription of double stranded RNA upon binding of an appropriate transcription factor to said promoters.

30

35

14. A method according to claim 12 wherein said nucleotide sequence is a part of or identical to said gene sequence conferring said phenotype, and which nucleotide sequence is such as to permit integration of said suitable or further vector by homologous recombination in the genome of said cell and following said integration said nucleotide sequence is capable of inhibiting expression of said gene sequence conferring said phenotype.

15. A method according to claim 14 wherein said nucleotide sequence comprises stop codons sufficient to prevent translation of said nucleotide sequence following its integration into said genome.

5

16. A method according to any preceding claim wherein said cell is a microorganism suitable for feeding to, transforming or infecting an organism.

10

17. A method according to any of claims 1 to 14 wherein said cell is contained in an organism or an embryo thereof.

15

18. A method according to any of claims 1 to 17 wherein said promoters are T7 promoters.

20

19. A method according to any of claims 12 to 18 wherein said known gene sequence comprises a sup-35 gene or a fragment thereof which is selectable by identifying offspring growing at a temperature above 25°C following introduction of said vector in the genome of a pha-1 et123ts mutant *C. elegans* worm.

25

20. A method according to any of claims 1 to 19 wherein said cell or organism is contacted with a specified compound for screening for a desired phenotype, such as resistance or sensitivity to said compound when compared to the wild type cell or organism.

30

21. A method according to any preceding claim wherein said transcription factor is inducible.

35

22. A method according to claim 16 wherein said microorganism is an *E. coli* strain which is an RNAase

Sheet 02

III and preferably an RNAase negative strain.

5

23. A method according to any of claims 17
wherein said organism is selected from the class
nematoda. | a

24. A method according to claim 23 wherein said
organism is *Caenorhabditis elegans*.

10

25. A method of generating a transgenic non-
human organism comprising an exogenous transcription
factor and a transgene comprising a promoter operably
linked to DNA fragment which is expressed upon binding
of said transcription factor thereto, the method
comprising

15

a) providing a first transgenic organism
comprising a first construct incorporating DNA
encoding an exogenous transcription factor and a
second transgenic organism comprising a second
construct including at least one promoter operably
linked to a desired DNA sequence which is expressed
upon binding of the transcription factor of said first
transgenic organism thereto,

20

25

b) crossing said first and second transgenic
organisms and selecting offspring expressing said
desired DNA sequence.

30

26. A method according to claim 25 wherein said
first and second transgenic organisms are generated by
transforming said first and second constructs into
respective microorganisms for subsequent feeding to
the respective organism.

35

27. A method according to claim 25 or 26 wherein
said second construct comprises said desired DNA | a

*Sub
ave
called*

sequence in an orientation relative to said promoter so as to be capable of initiating transcription of said DNA to dsRNA upon binding of said transcription factor thereto. a

5

28. A method according to claim 27 wherein said second construct comprises two promoters flanking said desired DNA sequence which promoters can initiate transcription of said DNA sequence to dsRNA upon binding of said transcription factor to said promoters.

10

29. A method according to claim 27 wherein said DNA sequence is provided in a sense and an antisense orientation relative to said promoter so as to produce dsRNA upon binding of the transcription factor to the promoter.

15

20

30. A method according to any of claims 25 to 29 wherein said second transgenic organism further comprises a reporter gene operably linked to a promoter which is capable of initiating transcription of said reporter upon binding of said transcription factor thereto.

25

31. A method according to any of claims 25 to 30 wherein said transcription factor comprises a polymerase. a

30

32. A method according to claim 31 wherein said polymerase comprises any of T7, T3 or SP6 polymerase.

35

33. A method according to any of claims 25 to 31 wherein said promoters comprises any of T7, T3 or SP6 promoters. a

34. A method according to claim 30 wherein said reporter gene comprises any of those sequence encoding Luciferase, Green Fluorescent protein, β galactosidase or β -lactamase.

5

35. A method according to any of claims 25 to 30 wherein said organism is of the species *nematoda*.

10

36. A method according to claim 35 wherein said *nematoda* species is *C. elegans*.

15

37. A transgenic non-human multicellular organism obtainable according to the methods of any one of claims 25 to 34.

10

38. A method of validating clones identified in yeast two hybrid vector experiments which method comprises

20

a) providing a construct including the DNA encoding the protein identified in the two hybrid vector experiment, which construct is such that said DNA is orientated relative to a promoter(s) that is capable of initiating transcription of said DNA to double stranded RNA upon binding of an appropriate transcription factor to said promoter(s),

25

b) transforming a cell comprising said transcription factor with said construct, and

30

c) identifying a phenotypic change in said cell or organism when compared to a wild type.

35

39. A method according to claim 38 wherein said DNA sequence is provided between two promoters capable of initiating transcription of the DNA sequence to dsRNA upon binding of the transcription factor to

said promoters.

40. A method according to claim 38 wherein said DNA is provided in a sense and an antisense orientation relative to said promoter such that binding of the transcription factor to said promoter initiates transcription of dsRNA from said DNA.

41. A method according to any of claims 38 to 40 wherein said transcription factor is inducible in said cell.

42. A method according to any of claims 38 to 41 wherein said promoter is a phage polymerase promoter and said transcription factor is a RNA polymerase.

43. A method according to claim 42 wherein said polymerase is any of T7 RNA polymerase, T3 RNA polymerase or SP6 RNA polymerase.

44. A method according to claim 43 wherein said promoters comprise any of T7, T3 or SP6 promoter

45. A method according to any of claims 38 to 44 wherein said construct is such that it may be used in yeast two hybrid experiments.

46. A method according to any of claims 38 to 45 wherein said cell is an E. coli cell.

47. A method according to any of claims 38 to 45 wherein said cell is part of an organism or an embryo thereof.

48. A method according to claim 47 wherein said

SVB
D7

organism is of the species nematoda and preferably *C. elegans*.

49. Plasmid pGN1 as illustrated in Figure 1.

50. Plasmid pGN100 as illustrated in Figure 2.

51. The yeast two hybrid vector plasmid
illustrated in any of Figures 4, 15 or 16 (Seq ID Nos 8
and 9).
a

52. A plasmid as illustrated in Figure 7.

53. A plasmid as illustrated in Figure 8.

54. A method of alleviating infestation of plant
pests, which method comprises

a) identifying a DNA sequence from said pest
which is critical for its survival, growth,
proliferation,

b) cloning said sequence from step a) or a
fragment thereof in a suitable vector in an
orientation relative to promoter(s) such that said
promoter(s) is capable of initiating transcription of
said DNA sequence to RNA or dsRNA upon binding of an
appropriate transcription factor to said promoter(s),
and

c) introducing said vector into the plant.

55. A method according to claim 54 wherein said
DNA sequence is provided between two promoters such
that binding of the transcription factor to the
promoters results in transcription of the DNA to
dsRNA.

56. A method according to claim 54 wherein said DNA sequence is provided in a sense and an antisense orientation relative to said promoter such that binding of the transcription factor to the promoter results in transcription of the DNA to dsRNA.

57. A method according to claim 54 wherein said pest is a nematode worm.

10 58. A method according to claim 57 wherein said nematode comprises any of *Tylenchulus* ssp., *Radopholus* ssp., *Rhadinaphelenchus* ssp., *Heterodera* ssp., *Rotylenchulus* ssp., *Pratylenchus* ssp., *Belonolaimus* ssp., *Canjanus* ssp., *Meloidogyne* ssp., *Globodera* ssp.,
15 *Nacobbus* ssp., *Ditylenchus* ssp., *Aphelenchoides* ssp., *Hirschmanniella* ssp., *Anguina* ssp., *Hoplolaimus* ssp., *Heliotylenchus* ssp., *Criconemella* ssp., *Xiphinema* ssp., *Longidorus* ssp., *Trichodorus* ssp., *Paratrichodorus* ssp., *Aphelenchs* ssp.

20 59. A method according to claim 55 wherein said DNA sequence or fragment thereof is cloned between two tissue, preferably root specific promoters.

25 60. An expression vector for use in a method according to any proceeding claim comprising a promoter or promoters oriented relative to a DNA sequence such that they are capable of initiating transcription of said DNA sequence to double stranded RNA upon binding of an appropriate transcription factor to said promoter or promoters.

30 35 61. An expression vector according to claim 60 comprising two identical promoters flanking the DNA sequence.

62. An expression vector according to claim 60 comprising said DNA sequence in a sense and an antisense orientation relative to said promoter.

5
63. An expression vector according to any of claims 60 to 62 which further comprises a nucleotide sequence encoding a selectable marker.

10
64. An expression vector according to claim 63 wherein said nucleotide sequence encoding said selectable marker is orientated relative to the promoter(s) such that transcription of the nucleotide sequence to double stranded RNA occurs upon binding of an appropriate transcription factor to said promoter(s).

15
20
65. An expression vector according to claim 64 wherein said nucleotide sequence encoding the selectable marker is provided between the identical promoters capable of initiating transcription of the nucleotide sequence to dsRNA upon binding of the transcription factor to the promoters.

25
30
66. An expression vector according to claim 64 wherein said nucleotide sequence encoding the selectable marker is provided in a sense and an antisense orientation relative to the promoter such that transcription of the nucleotide sequence to dsRNA upon binding of the transcription factor to said promoter occurs.

35
67. An expression vector according to claim 63 or 64 wherein said selectable marker comprises a nucleotide sequence encoding sup-35, for introduction into *C. elegans* having a pha-1 mutation.

Feb 13 1987
5

68. An expression vector for expressing an appropriate transcription factor for use in a method according to any of claims 1 to 48 and 54 to 59 which vector comprises a sequence of nucleotides encoding said transcription factor operably linked to suitable expression control sequences. *a*

10 69. An expression vector according to claim 68 wherein said expression control sequences include promoters which are inducible, constitutive, general or tissue specific promoters, or combinations thereof.

15 70. An expression vector according to any of claims 68 to 69 wherein said transcription factor comprises a phage polymerase, and preferably T7 RNA polymerase.

20 71. An organism or cell transformed or transfected with a plasmid according to any of claims 49 to 53 or an expression vector according to any of claims 60 to 70.

25 72. An organism according to claim 71, which is of the species nematoda and preferentially *C. elegans*.

30 73. A method of introducing dsRNA or DNA capable of producing dsRNA into an organism which method comprises feeding said organism with a suitable microorganism comprising an expression vector according to any of claims 60 to 67 or feeding said organism directly with an expression vector according to any of claims 60 to 67. *a*

35 74. A method according to claim 73 wherein said microorganism or said organism is adapted to express

said transcription factor.

75. A method according to claim 74 wherein
either said microorganism or said organism comprises
5 an expressing vector according to any of claims 66 to
69.

76. A method according to any of claims 73 to 75
wherein said organism is *C. elegans* and said
microorganism is *E. coli*. *a*

77. A method according to claim 76 wherein said
E. coli strain is an RNAaseIII negative strain.

15 78. A method according to any of claims 73 to 75
wherein said organism is a *C. elegans* nuc-1 mutant.

20 79. A selection system for identifying
transformation of a cell or organism with a vector
according to claims 60 to 63 which system comprises a
vector according to claims 60 to 63 and said
selectable marker comprises a nucleotide sequence
capable of inhibiting or preventing expression of a
gene in said cell or organism which gene is
25 responsible for conferring a known phenotype. *a*

30 80. A selection system according to claim 79
wherein said nucleotide sequence comprises a sequence
which is a part of or identical to said gene
conferring said known phenotype, and which nucleotide
sequence is itself located between two identical
promoters capable of initiating transcription of
double stranded RNA upon binding of an appropriate
transcription factor to said promoters.

81. A selection system according to claim 79 wherein said nucleotide sequence is a part of or identical to said gene sequence which confers a known phenotype on said cell or organism, and which 5 nucleotide sequence permits integration of said vector by homologous recombination in the chromosome of said cell or organism and following said integration said sequence inhibits expression of said gene sequence conferring said known phenotype.

10

82. A selection system according to claim 81 wherein said nucleotide sequence comprises stop codons sufficient to prevent translation of said nucleotide sequence following integration into said genome.

15

83. A selection system according to claim 79 wherein said known gene sequence comprises a sup-35 gene or a fragment thereof which is selectable by identifying offspring growing at a temperature above 20 25°C following introduction of said vector in a pha-1 et123ts mutant *C. elegans* worm.

84. A method according to claim 74 wherein said transcription factor is T7 RNA polymerase.

25

85. A method of assigning function to a DNA sequence of a multicellular organism which method comprises:

a) providing:

30 (i) a first construct comprising said DNA sequence cloned in a sense direction under the regulation of a suitable promoter

(ii) a second construct comprising said DNA sequence cloned in an anti-sense direction under the 35 regulation of the same promoter as in step (i),

in a multicellular organism which is adapted to initiate transcription of said DNA fragment from said promoter,

5 b) identifying the phenotype of said multicellular organism compared to wild type.

86. A method of assigning function to a DNA sequence of a multicellular organism which method comprises:

10 a) providing
 i) a construct comprising said DNA fragment cloned between two promoters capable of promoting transcription in said multicellular organism, in a multicellular organism capable of
15 initiating transcription from said promoter;
 b) identifying the phenotype of said multicellular organism compared to wild type.

87. A method for expressing a gene, cDNA or DNA fragment in *C. elegans* which method comprises :

20 i) providing a transgenic *C. elegans* expressing an exogenous transcription factor,
 ii) cloning said gene or cDNA or DNA fragment into an appropriate vector operably linked to a
25 promoter capable of initiating transcription of said gene, cDNA or DNA fragment upon binding of said exogenous transcription factor thereto,
 iii) introducing said vector into said transgenic *C. elegans* from step i).

30 88. A method according to claim 87 wherein said exogenous transcription factor is a phage polymerase.

35 89. A method according to claim 88 wherein

said phage polymerase is T3, SP6 or T7 RNA polymerase.

90. A method according to any of claims 87 to
89 wherein said promoter is any of T3, T7 or SP6
promoter. *a*

ADD 7
DS 7